JOURNAL OF CLINICAL MICROBIOLOGY, May 1994, p. 1135–1141
0095-1137/94/$04.00+0

Vol. 32, No. 5

# Epidemiologic Analysis of Sporadic *Salmonella typhi* Isolates and Those from Outbreaks by Pulsed-Field Gel Electrophoresis

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Received 14 October 1993/Returned for modification 24 November 1993/Accepted 24 January 1994

**Pulsed-field gel electrophoresis (PFGE) was used to compare and analyze 158 isolates of *Salmonella typhi* from five well-defined outbreaks of typhoid fever in Malaysia and also isolates involved in sporadic cases of typhoid fever occurring during the same period. Digestion of chromosomal DNAs from these *S. typhi* isolates with the restriction endonucleases *Xba*I (5′-TCTAGA-3′), *Spe*I (5′-ACTAGT-3′), and *Avr*II (5′-CCTAGG-3′) and then PFGE produced restriction endonuclease analysis (REA) patterns consisting of 11 to 24 DNA fragments ranging in size from 20 to 630 kbp. Analysis of the REA patterns generated by PFGE after digestion with *Xba*I and *Spe*I indicated that the *S. typhi* isolates obtained from sporadic cases of infection were much more heterogeneous (at least 13 different REA patterns were detected; Dice coefficient, between 0.73 and 1.0) than those obtained during outbreaks of typhoid fever. The clonal nature and the close genetic identities of isolates from outbreaks in Alor Setar, Penang, Kota Kinabalu, Johor Bahru, and Kota Bahru were suggested by the fact that only a limited number of REA patterns, which mostly differed by only a single band, were detected (one to four patterns; Dice coefficient, between 0.82 and 1.0), although a different pattern was associated with each of these outbreaks. Comparison of REA patterns with ribotyping for 18 *S. typhi* isolates involved in sporadic cases of infection showed a good correlation, in that 72% of the isolates were in the same group. There was no clear correlation of phage types with a specific REA pattern. We conclude that PFGE of *S. typhi* chromosomal DNA digested with infrequently cutting restriction endonucleases is a useful method for comparing and differentiating *S. typhi* isolates for epidemiological purposes.**

In many developing countries in the tropical parts of the world, typhoid fever remains an important cause of morbidity and mortality, with an estimated annual global incidence of 21 million cases and more than 700,000 deaths. In Malaysia, the disease is endemic, and its incidence appears to be increasing, with periodic outbreaks occurring recently in various parts of the country (5). The emergence of antibiotic-resistant strains of *Salmonella typhi* (13) and the increased incidence of typhoid fever in human immunodeficiency virus type 1-infected individuals are further causes for concern. In relation to effective surveillance and the development of rational control strategies for this important human disease, the availability of detailed and accurate data related to the molecular epidemiology of *S. typhi* is crucial. However, epidemiological investigations used to determine the source and spread of *S. typhi* have been hampered by the absence of reliable and sufficiently discriminative methods of differentiating individual strains beyond the species level. Methods that have been used include antibiotic resistance patterns, biochemical reactions, phage typing, and plasmid analysis. More recently, there has been an increasing interest in the application of molecular techniques to type bacterial pathogens (35). With *S. typhi*, techniques such as multilocus enzyme electrophoresis (27, 32), lipopolysaccharide (16) and envelope protein profiles (11), chromosomal restriction endonuclease digestion patterns (11), and ribotyping (1, 24) have been used. Many of these techniques are not sufficiently sensitive for distinguishing individual strains and investigators often find them tedious to perform. The recent development of pulsed-field gel electrophoresis (PFGE) (31, 33, 34) has provided another approach for obtaining molecular fingerprints which may be useful in epidemiological studies (22). PFGE has been used successfully to perform comparative chromosomal DNA analysis of several bacterial pathogens for epidemiological investigations (8, 10, 12, 14, 15, 20, 23, 26, 29, 30) and is believed to possess a discriminating capacity greater than those of ribotyping and other probe-based restriction fragment length polymorphism methods (35). The restriction endonuclease analysis (REA) patterns generated by PFGE generally consist of a fairly small number of well-separated bands which present fewer difficulties of interpretation and ambiguities compared with the patterns generated by conventional agarose gel electrophoresis. PFGE is also useful for assessing the extent of molecular diversity within a species, physical and genetic mapping of bacterial chromosomes, and estimating genome sizes (28). We report here that PFGE following *Xba*I and *Spe*I restriction digestion of *S. typhi* chromosomal DNA is a useful method for differentiating individual isolates of *S. typhi* and in the molecular analysis of isolates involved in outbreaks of typhoid fever. To our knowledge, this represents the first study in which a wide variety of epidemiologically distinct *S. typhi* isolates have been studied by PFGE.

## MATERIALS AND METHODS

**Bacterial strains.** Isolates of *S. typhi* from either the blood or stools of humans were used in the present study. The organisms were isolated, maintained, and identified by standard methods (7). Multiple isolates were obtained during well-documented outbreaks in various parts of Malaysia between

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1987 and 1991 (5) (Table 1). In addition, isolates from sporadic cases of typhoid fever were also obtained during the same period (but not during the outbreak periods) and in approximately the same geographical locations (Table 1). Vi phage typing of the isolates was performed by standard procedures by the Salmonella Reference Centre at the Institute for Medical Research, Kuala Lumpur, Malaysia. Repeated subculturing of isolates was avoided, and stocks of the primary isolates were maintained at −70°C. All *S. typhi* isolates tested were susceptible to ampicillin, chloramphenicol, kanamycin, streptomycin, co-trimoxazole, and tetracycline, as determined by standard disk diffusion procedures for measuring resistance (7).

**Preparation of DNA.** DNA for PFGE analysis was prepared by a modification of the method of Smith et al. (34). To prepare chromosomal DNA, bacterial cells were grown with shaking at 37°C in nutrient broth to an optical density at 650 nm of 0.4. Cells were then centrifuged, washed with 10 mM Tris-HCl (pH 7.5)–1 M NaCl, and mixed with an equal volume of molten 1.5% low-melting-point agarose (InCert agarose; FMC Bioproducts, Rockland, Maine). Agarose blocks were incubated overnight at 37°C in lysozyme solution (2 mg of lysozyme per ml in 6 mM Tris-HCl, 1 mM NaCl, 100 mM EDTA, 0.5% Brij, 0.22% deoxycholate, 0.5% Sarkosyl, and 2 μg of RNase per ml); this was followed by deproteination (in 1 mg of proteinase K per ml in 0.5 M EDTA and 0.5% Sarkosyl) at 50°C for 48 h. The cell debris and proteinase K were then removed by two washes in 5 ml of 10 mM Tris-HCl–0.1 mM EDTA (TE buffer) containing 1 mM phenylmethylsulfonyl fluoride (Sigma Chemical Co., St. Louis, Mo.) for 2 h at room temperature. DNA plugs were then equilibrated in TE buffer for 2 h at room temperature.

**Restriction endonuclease digestion and PFGE.** Before restriction enzyme digestion, agarose blocks were first equilibrated for 2 h in 200 μl of the appropriate buffer. Fresh buffer (containing the enzyme at 8 to 20 U/μg of DNA) was then added, and the blocks were incubated overnight at various temperatures with gentle agitation (*Xba*I, 37°C; *Avr*II, 37°C; *Spe*I, 4°C overnight and then at 37°C for 12 h). Selection of restriction enzymes was based on the recognition site of the enzyme and the G+C content of 50 to 54% previously reported for *Salmonella* spp. (17). The following restriction endonucleases were used: *Xba*I (5′-TCTAGA-3′), *Avr*II (5′-CCTAGG-3′), and *Spe*I (5′-ACTAGT-3′) (New England Biolabs, Beverly, Mass., and Stratagene Co., La Jolla, Calif.). PFGE of inserts was performed by the contour-clamped homogeneous electric field method on a CHEF-DR II system (Bio-Rad Laboratories, Richmond, Calif.) in gels of 1% agarose in 0.5× TBE buffer (0.1 M Tris, 0.1 M boric acid, 0.2 mM EDTA) for 28 to 30 h at 200 V at a temperature of 14°C, with ramped pulse times varying according to the enzymes used (ranging from 2 to 50 s). The gels were stained with ethidium bromide and were photographed with a UV transilluminator (Spectroline; 302 nm). The DNA size standards used were a bacteriophage lambda ladder consisting of concatemers starting at 48.5 kbp and increasing to approximately 1,000 kbp (Bio-Rad Laboratories, Richmond, Calif.) and a mid-range II PFG Marker (size range, 24 to 291 kbp; New England Biolabs). Determination of the sizes of the DNA fragments was carried out by measuring the migration distances of individual bands and referring these measurements to a standard curve which plotted the migration distances against the logarithm of the molecular size of the DNA size standards. For each strain tested with various restriction endonucleases, between 11 and 24 bands were usually seen, and all visible bands were included in determining the REA patterns. The existence of doublets (comigrating bands) was also taken into account by referring to the band intensity. Several electrophoretic runs with ramped pulse times varying between 2 and 50 s were performed to obtain the optimal times in which individual bands were clearly separated.

**Data analysis.** DNA fragment patterns were visually assessed, and distinct patterns were assigned an arbitrary REA pattern. Isolates were considered to be genetically similar or identical if there was complete concordance of the DNA fragment profiles and were considered different if there was a difference of one or more DNA bands. The REA patterns generated by PFGE for various isolates were compared, and the similarities of the fragment length patterns between two strains were scored by the Dice coefficient (28), also known as a coefficient of similarity (8). This coefficient, $F$, expresses the proportion of shared DNA fragments in two isolates and was calculated by the following formula: $F = 2n_{xy}/(n_x + n_y)$, where $n_x$ is the total number of DNA fragments from isolate X, $n_y$ is the total number of DNA fragments from isolate Y, and $n_{xy}$ is the number of DNA fragments that were identical in the two isolates. An $F$ value of 1.0 indicates that the two isolates have identical REA patterns.

**Ribotyping.** Selected isolates were subjected to ribotyping analysis to obtain rRNA gene restriction patterns as described previously (24).

## RESULTS

A total of 158 isolates of *S. typhi* obtained during defined outbreaks or from sporadic cases of typhoid fever were studied (Table 1). Nine different phage types were represented among these isolates (Table 1), and all isolates were susceptible to ampicillin, chloramphenicol, kanamycin, streptomycin, co-trimoxazole, and tetracycline. Of the 158 isolates studied, 56 (35%) were phage type D1 and 54 (34%) were type E1 (Table 1).

On the basis of the reported 50 to 54% G+C content of *Salmonella* spp., a number of restriction endonucleases were screened to determine which ones gave suitable numbers of well-separated fragments. Three enzymes (*Xba*I, *Spe*I, and *Avr*II) gave the optimum number of fragments and the clearest REA patterns. PFGE analysis of the digested DNAs yielded between 11 and 24 fragments ranging in size from 20 to 630 kbp (Fig. 1 to 3). Several other enzymes tested (e.g., *Ase*I, *Pne*I, *Sma*I, *Bgl*I, and *Pvu*I) produced too many fragments of <50 kbp, which were difficult to separate by PFGE (data not shown).

The REA patterns generated by PFGE after *Xba*I digestion for 18 individual *S. typhi* isolates (from sporadic cases; Table 1) were compared with the REA patterns obtained previously with an rRNA gene probe (24). Both approaches produced eight different patterns, and 13 of 18 isolates (72%) followed the same grouping (Table 2; Fig. 4). Results also show that there is no clear correlation between the REA patterns generated by PFGE with the phage type of a particular isolate, because different phage types can have similar PFGE patterns (Table 2).

The restriction endonucleases *Xba*I and *Spe*I were then used to perform PFGE analysis on *S. typhi* isolated during several recent outbreaks of typhoid fever in Malaysia as well as from sporadic cases occurring in the same period and geographical location. Among the sporadic isolates, patterns which were distinctly different from one another could clearly be seen (Fig. 1). Representative results in Fig. 1A (for digestion with *Xba*I), for example, show eight different REA patterns among the eight isolates tested. Figure 1B (for digestion with *Spe*I) shows eight different patterns among nine isolates. At least 13

TABLE 1. Isolates of *S. typhi* tested for Vi phage type and REA patterns by PFGE following digestion with *Xba*I and *Spe*I

| Location and date (mo/yr) of outbreak | No. of *S. typhi* isolates tested | Phage type (no. of isolates) | No. of REA patterns[a] (*F* value) | |
|---|---|---|---|---|
| | | | *Xba*I | *Spe*I |
| Alor Setar (5/1990) | 20 | E1 | 1 (1.0) | 1 (1.0) |
| Penang (6/1987–9/1987) | 21 | D1 | 2 (0.97–1.0) | 2 (0.97–1.0) |
| Kota Kinabalu (3/1987) | 22 | E1 | 2 (0.93–1.0) | 1 (1.0) |
| Johor Bahru (4/1990–5/1990) | 20 | D1 | 2 (0.97–1.0) | 3 (0.94–1.0) |
| Kota Bahru (10/1991) | | B1 (15)<br>E1 (3)<br>C4 (2) | 4 (0.84–1.0) | 4 (0.82–1.0) |
| Sporadic cases (1987–1991) | 48 | D1 (14)<br>E1 (9)<br>A (7)<br>D2 (7)<br>B1 (3)<br>53 (1)<br>D9 (1)<br>C10 (1)<br>unt (5)[b] | ≥13 (0.73–1.0) | ≥13 (0.74–1.0) |

[a] The proporation of isolates with different patterns (P) are as follows: for isolates from Penang, *Xba*I, P1 = 83%, P2 = 17%; *Spe*I, P1 = 94%, P2 = 6%; for isolates from Kota Kinabalu, *Xba*I, P1 = 82%, P2 = 18%; for isolates from Johor Bahru, *Xba*I, P1 = 90%, P2 = 10%; *Spe*I, P1 = 80%, P2 = 10%, P3 = 10%; for isolates from Kota Bahru, *Xba*I, P1 = 75%, P2 = 6%, P3 = 6%, P4 = 13%; *Spe*I, P1 = 64%, P2 = 12%, P3 = 12%, P4 = 12%.

[b] unt, untypeable.

different REA patterns were noted among the 48 isolates tested (Table 1), with *F* values ranging from 0.73 to 1.0 (Table 1). In contrast, the isolates from an outbreak of typhoid fever in Alor Setar appeared to be genetically identical following digestion with both enzymes (Table 1) (*F* = 1.0). Isolates from the Penang outbreak showed two REA patterns (Table 1) with both enzymes, differing by a single band and giving *F* values of between 0.97 and 1.0 (Table 1; Fig. 2 and 3). Similarly, isolates from the Kota Kinabalu outbreak showed a single clone with *Spe*I (*F* = 1.0), but two REA patterns, differing by a single band, were observed by *Xba*I digestion (*F* = 0.93 to 1.0) (Table 1; Fig. 2 and 3). The *S. typhi* isolates from the outbreak in Johor Bahru showed two REA patterns with *Xba*I (*F* = 0.97 to 1.0) and three REA patterns with *Spe*I (*F* = 0.94 to 1.0) (Table 1); these patterns differed by one to two bands. With the isolates obtained from outbreaks in Penang, Kota Kinabalu (with *Xba*I), and Johor Bahru, in which more than one REA pattern was observed by PFGE, it was found that one particular pattern predominated and the extra pattern(s) was associated with only a few isolates (Table 1). The outbreak in Kota Bahru was associated with three phage types of *S. typhi* possessing four different REA patterns following digestion with both *Xba*I and *Spe*I (*F* = 0.82 to 1.0) (Table 1; Fig. 2 and 3). Comparison of representative isolates from these five outbreaks on a single gel indicated that a different REA pattern was associated with each of these outbreaks. The REA patterns generated by PFGE were both stable and reproducible when repeated analysis of these strains was performed.

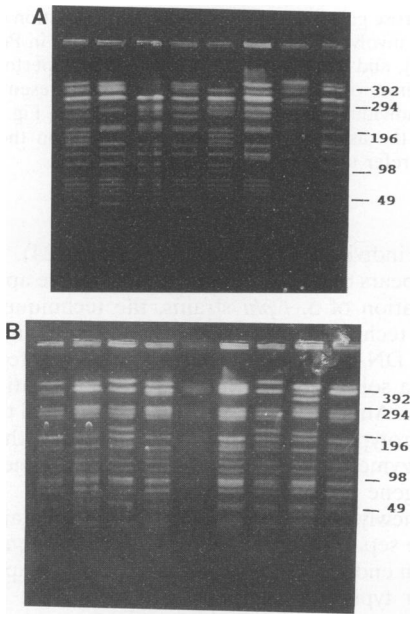


FIG. 1. Agarose gel showing the *Xba*I (A) and *Spe*I (B) digestion patterns of *S. typhi* isolates involved in sporadic cases of typhoid fever in Malaysia (see Table 1). PFGE was performed with ramped pulse times of 2 to 40 s (*Xba*I) and 1 to 40 s (*Spe*I). Individual lanes represent the REA patterns for individual isolates. Values on the right (in kilobase pairs) refer to the positions of marker bands.

## DISCUSSION

Typhoid fever continues to be a major public health problem in developing countries as a result of many interrelated factors, including increased urbanization, inadequate supplies of clean water, antibiotic resistance, the variable efficacies of vaccine preparations, and the increased regional movements of large numbers of migrant workers. The last factor is especially important in the Asia-Pacific region because of the rapid pace of economic development. It is thus obvious that there is an urgent need for effective epidemiological surveillance. Therefore, specific and reliable epidemiological markers for *S. typhi*

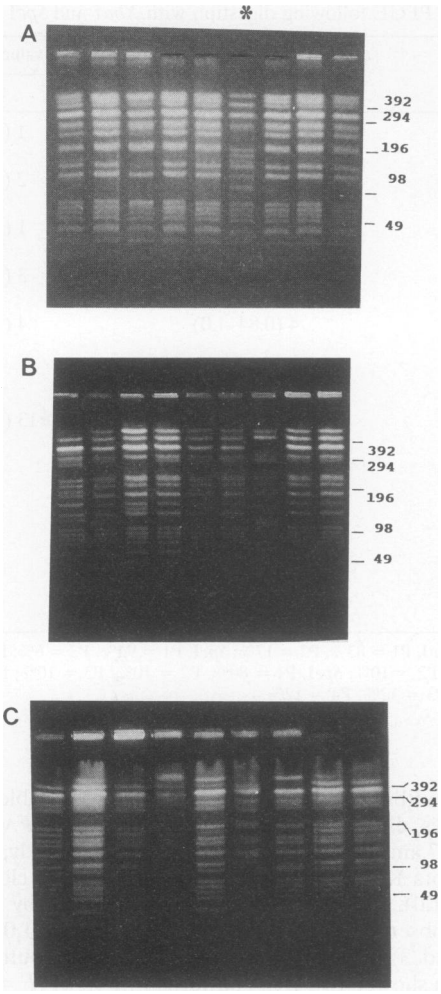


FIG. 2. Agarose gel showing showing the *Xba*I digestion patterns of *S. typhi* isolates involved in outbreaks of typhoid fever in Penang (A), Kota Bahru (B), and Kota Kinabalu (C). PFGE was performed with ramped pulse times of 2 to 40 s. Individual lanes represent the REA patterns for individual isolates. The asterisk in panel A indicates an *S. typhi* isolate involved in a sporadic case of typhoid fever which was included in the gel for comparative purposes. Values on the right (in kilobase pairs) refer to the positions of marker bands.

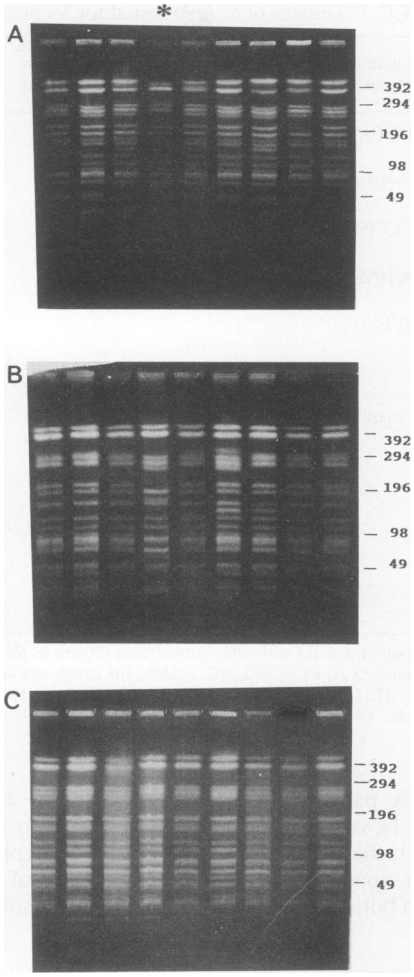


FIG. 3. Agarose gel showing showing the *Spe*I digestion patterns of *S. typhi* isolates involved in outbreaks of typhoid fever in Penang (A), Kota Bahru (B), and Kota Kinabalu (C). PFGE was performed with ramped pulse times of 1 to 40 s. Individual lanes represent the REA patterns for individual isolates. See the legend to Fig. 2 for an explanation of the asterisk above panel A. Values on the right (in kilobase pairs) refer to the positions of marker bands.

are required. The differentiation of individual *S. typhi* strains has been problematic in the past. Plasmid profiles are not very useful, because only a small proportion (<10%) of strains harbor plasmids (25). Vi phage typing has been of some value, but it is technically difficult and can be performed only by reference laboratories. In addition, a significant proportion of strains (22%) are not typeable and some are Vi negative (6). Analysis of the envelope protein profiles of *S. typhi* strains isolated in Peru and Indonesia detected only minor differences between strains (11). Multilocus enzyme electrophoresis analysis indicated that all *S. typhi* strains analyzed had identical isoenzyme patterns, thus suggesting that they belong to a single clone (27). However, later studies suggested that a few different electrophoretic types can be defined by multilocus enzyme electrophoresis (32). Studies of lipopolysaccharide profiles also suggested that heterogeneity exists among *S. typhi* strains (16). More recently, analysis of rRNA gene restriction patterns (ribotyping) indicated that considerable genetic heterogeneity exists among individual strains of *S. typhi* (1, 24). Although ribotyping appears to be a sensitive, discriminative approach to the differentiation of *S. typhi* strains, the technique requires considerable technical expertise involving the digestion of chromosomal DNA with restriction enzymes, transfer of DNA fragments to a solid support, and finally, hybridization with a labelled probe. Another recent study (18) suggested that major *Salmonella* serotypes can also be differentiated on the basis of restriction enzyme digestion of an amplified fragment of the *fliC* flagellin gene produced by PCR.

PFGE, a newly developed method in which large DNA fragments are separated after digestion with infrequently cutting restriction endonucleases, has provided a new approach to the molecular typing of bacterial pathogens (22). The technique has been used successfully for molecular epidemiological analyses of a variety of bacterial species. For example, PFGE was found to be a useful method for investigating the source, transmission, and spread of nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* (8, 26), *Mycobacterium fortuitum* (14), and *Pseudomonas cepacia* (2). It has also

TABLE 2. Comparison between REA patterns from PFGE following digestion with *Xba*I and ribotyping for 18 *S. typhi* isolates

| REA pattern by PFGE | | REA pattern by ribotyping | |
|---|---|---|---|
| Group | Strain no.[a] | Group | Strain no. |
| A | 2 (B1), 107 (D1) | 1 | 2, 4 (D2), 103 (B1) |
| B | 103 | 6 | 105 (53) |
| C | 3 (B1), 5 (D1), 10 (D1) | 2 | 3, 5, 10 |
| D | 7 (D2) | 4 | 7, 110 (D2) |
| E | 6 (D1), 8 (A), 9 (D9), 112 (D1), 113 (D1), 104 (A), 105, 110 | 3 | 6, 8, 9 112, 113, 107, 108 (unt[b]) |
| F | 11 (C10) | 5 | 11, 104 |
| G | 4 | 7 | 109 (unt) |
| H | 111 (D1) | 8 | 111 |

[a] Phage types are given in parentheses. Pattern B differs from pattern A by a single band; hence, isolate 103 is categorized in the same PFGE pattern group as isolates 2 and 107 for the purpose of calculating the *F* value of 72% between PFGE and ribotyping referred to in the text.

[b] unt, untypeable.

been used in molecular epidemiological studies with group B streptococci (10), *Streptococcus pneumoniae* (20), *Enterococcus faecium* (23), and rickettsiae (9). In a study in which 60 *S. aureus* isolates were typed by 15 investigators at 10 different institutions by using all of the commonly used typing methods, PFGE and other DNA-based techniques were most effective in correctly typing the isolates (36). In the present study, we found a good correlation between PFGE and ribotyping with regard to the ability to differentiate and group *S. typhi* isolates. Studies with other bacteria have, in fact, shown PFGE to be superior to ribotyping in strain differentiation (12, 26, 30), in that PFGE is able to subdivide ribotypes (30). PFGE was also superior to a random polymorphic DNA assay in differentiating methicillin-resistant *S. aureus* (29). The REA patterns produced by PFGE are stable and reproducible and are relatively easier to interpret compared with the patterns produced by separation of digested DNA by conventional agarose gel electrophoresis, because of the smaller number of fragments generated. With regard to the enzymes which generate informative REA patterns for genome fingerprinting, Römling et al. (28) have found that enzymes with a hexameric recognition sequence which contains CTAG often turn out to be most useful, because CTAG is counterselected in most bacterial genomes (28). The enzymes used in the present study, *Xba*I, *Spe*I, and *Avr*II, all have hexameric recognition sequences containing CTAG. Compared with ribotyping, PFGE is more convenient to perform because it does not involve the transfer of DNA fragments from agarose gels to solid supports (e.g., nylon or nitrocellulose membranes). It is also less hazardous because the requirement to subsequently probe the membrane with a radioactively labelled probe is obviated. Furthermore, the REA pattern differences generated by PFGE are a reflection of variation over the entire genome of *S. typhi* rather than polymorphisms within a specific gene cluster detected with a probe (1, 24). Of the many variants of PFGE currently in use, it has been shown that the contour-clamped homogeneous electric field electrophoresis system used in the present study is one of the systems which meets the criteria for reliable macrorestriction fragment pattern analysis (26). PFGE will also be useful in future studies for constructing physical and genetic maps of the *S. typhi* chromosome, as has been illustrated with other bacterial species (3, 19, 21, 37).

The present analysis of *S. typhi* by PFGE also provided some valuable insights into the epidemiology of typhoid fever in Malaysia. It is clear from the data that considerable heterogeneity exists at the DNA level among *S. typhi* isolates from an endemic area. The results also suggest that multiple clones of *S. typhi* are endemic to Malaysia and coexist simultaneously, causing sporadic cases of typhoid fever among the population throughout the year. It is also clear that most of the periodic outbreaks of typhoid fever which occur in Malaysia from time to time (5) are caused by a single clone or very closely related clones with very similar REA patterns, although no single clone(s) has been found to be associated with all outbreaks. The present study does not, however, enable us to make any conclusions with regard to whether distinct strains are responsible for a particular outbreak or whether the outbreaks are caused by the same strain(s) which has undergone a mutation in a particular site for a restriction endonuclease. It has also been argued that strains with very similar PFGE patterns (differing in only one or two bands) are clonally related (22). Given the sensitivity and discriminatory power of PFGE, minor differences in REA patterns are to be expected even among strains from a well-defined common-source outbreak (22), and a less than 100% match should be accepted when considering these strains to be clonally related. We also found that when two to three REA patterns were observed among the isolates from a particular outbreak, one pattern was associated with the majority of isolates and the additional pattern(s) (differing from the predominant pattern by one or two bands) was present in only a small minority of the isolates. The results also suggest that the ability of *S. typhi* to cause outbreaks or epidemics is not confined to any one genetic type or clone of *S. typhi*. The validity of the conclusions made about the association of REA patterns with the epidemiological characteristics of these *S. typhi* isolates was suggested by the fact that both of the restriction endonucleases used in the present study, *Xba*I

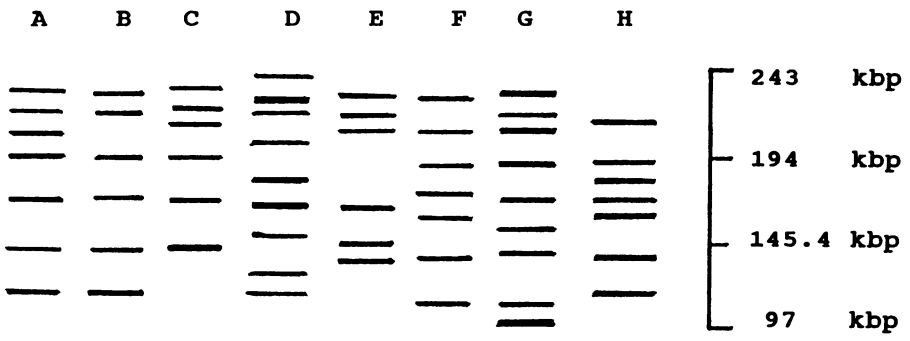


FIG. 4. Diagrammatic representation of the REA patterns by PFGE presented in Table 2.

and *Spe*I, gave the same results. The present study also found no obvious correlation of REA patterns with phage types. The most common phage types detected in the present study were D1 and E1, which is in close agreement with a previous report, in which 15.8% of all isolates were the D1 type and 19.3% were the E1 type (6). For comparative purposes, it would also be of interest to study *S. typhi* from environmental sources (e.g., surface waters and food), although it has been reported that such isolates are difficult to obtain, even in endemic areas (4).

The findings of the present study with *S. typhi* strains isolated from an endemic area suggest that PFGE is a useful technique for differentiating individual strains and may complement phage typing and other molecular approaches for epidemiological purposes. It is now perhaps timely to evaluate the use and applications of molecular typing methods for typing bacteria of public health importance (28, 35). In view of the increasing disease activity on a global level, application of these methods should not be limited to those pathogens which cause nosocomial infections (2, 8, 15, 30), but perhaps should also include those associated with epidemics, such as *S. typhi* and *Vibrio cholerae*. Once potentially useful and promising methods are standardized with regard to protocols and methodologies, data analysis, and interpretation, international databases could perhaps be set up. These databases could be used for effective epidemiological surveillance and the prevention and control of important bacterial infections.

## ACKNOWLEDGMENTS

The research described in this report was funded by IRPA grants 3-07-04-084 and 3-07-04-026 from the Ministry of Science, Technology & Environment, Malaysia, and grant 30331B from the National Working Group on Biotechnology, National Council for Scientific Research & Development, Ministry of Science, Technology & Environment, Malaysia.

We thank K. J. Forbes of the Department of Medical Microbiology, University of Aberdeen, Aberdeen, United Kingdom, for invaluable assistance in establishing the PFGE technique in our laboratory and Antonius Suwanto, Bogor Agricultural University, for useful discussions.

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